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Synthesis of Symmetrical 1,5-Bisacyloxyanthraquinone Derivatives and Their Dual Activity of Cytotoxicity and Lipid Peroxidation

Symmetrical bis-substituted anthraquinones were successfully prepared and demonstrated potent cytotoxicity against the growth of suspended murine and human tumors, i.e. rat glioma C6 cells and human hepatoma G2 cells. We report here a convenient synthetic pathway that leads to symmetrically substituted 1,5-bisacyloxyanthraquinone derivatives. Acylation of the hydroxyl group of 1,5-dihydroxyanthraquinone with the appropriate acyl chlorides in the presence of pyridine or sodium hydride, respectively, furnished this structural class of anthraquinones. The bis(butyryloxy) analog **2b**, bis(2-chlorobenzoyl) analog **2f**, and bisphenylpropionyloxy analog **2n** exhibit potent cytotoxicity in inhibition of human hep G2 cell growth in culture, as determined by using XTT colorimetric assay, while their antiproliferative activity is markedly enhanced and is comparable to that of the anticancer agent mitoxantrone. In addition, redox properties of the compounds for the inhibition of lipid peroxidation in model membranes were determined. Compounds **2n** also exhibited stronger antioxidant activity than ascorbic acid, (+)-α-tocopherol, and anthrarufin. Biological evaluation and SAR studies of these symmetrical anthraquinones have been performed and the results are discussed.

Keywords: Anthraquinone; Cytotoxicity; Rat glioma C6 cells; Human hepatoma G2 cells; XTT colormetric assay; Lipid peroxidation

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Introduction

A number of analogs of anthraquinone have been synthesized and evaluated both for preclinical antitumor efficacy as well as for biochemical pharmacology [1–5]. Intercalating agents continue to occupy a prominent position in the treatment of malignant diseases and thus the antitumor and biochemical effects of these compounds remain subjects of intensive research. The anthraquinone (anthracene-9,10-dione) mitoxantrone has been shown to have outstanding antitumor activities but a much narrower spectrum of activity in comparison with those of the anthracyclines [3]. Anthracyclines and anthraquinones form ternary complexes with DNA and the enzyme and stimulate DNA cleavage in a sequence-specific manner [6]. Consequently, considerable effort has been invested in the development of structural analogs or other classes of DNA binders which may circumvent or at least reduce this disadvantage. Based on these facts, there are still demands for synthesis of new analogs of anthraquinone to provide good antitumor activities and less toxic side effects. On the other hand, management of antitumor therapy is necessarily complementary and antioxidation is one of the most interesting aspects investigated in this context. Previously, we have developed series of structurally related substituted 1,5-and 1,8-dichloroanthracenes as potential antitumor analogs. The positional attachment of the side chain has been shown to profoundly influence their activity. Therefore, the need still exists for anthraquinone or anthracene congeners endowed with improved therapeutic efficacy and less toxic side effects, as well as effectiveness against multiple drug-resistant (MDR) cell lines [7]. As in the case of the anthracyclines, the mechanisms of cell kill by the anthraquinones are poorly understood. The goal of the present study was an evaluation of the importance of the side-arm substitution patterns at the 1 and 5 positions of the anthraquinone skeleton: not only would the nature of the side arms influence the binding or kinetic dissociation rate of the anthraquinone-DNA intercalant but they might also be intimately involved in lipid peroxidation. This has enabled us to address the issue of how, and to what extent, the position of side chain substituents affects biological activity.

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Results

The synthesis of 1,5-bisacyloxyanthraquinone derivatives shown in Figure 1 was accomplished using proce-

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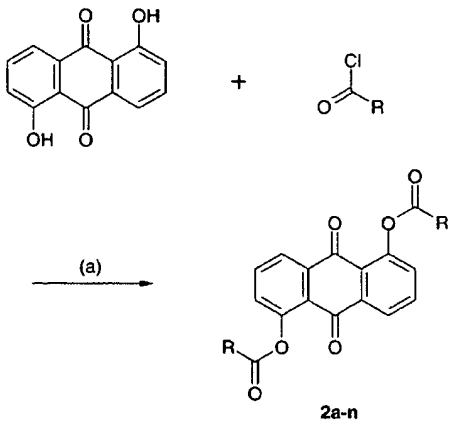


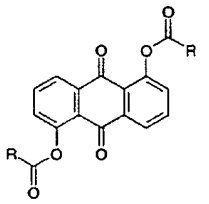
Figure 1. Reagents: (a) Method A, pyridine, CH_2Cl_2, N_2, R = $(CH_2)_nCH_3$, n = 1,2,5; C_6H_5; 2-$CH_3C_6H_4$; 4-$CH_3C_6H_4$; $CH_2C_6H_5$; Method B, NaH, THF, R = $C(CH_3)_3$; 2-ClC_6H_4; 3-ClC_6H_4; 4-ClC_6H_4; 2,4-$Cl_2C_6H_3$; 3-$CH_3C_6H_4$.

dures somewhat modified from those described elsewhere [8–12]. The method of preparation of the symmetrical bis-substituted anthraquinones was based on that of simple acylation involving 1,5-dihydroxyanthraquinone (anthrarufin) with an excess of the appropriate acyl chlorides in the presence of pyridine and dichloromethane at room temperature for 1 to 2 hours; or in the presence of NaH and THF at room temperature or under reflux for 1 to 2 hours. Accordingly, acylation of the appropriate anthraquinones with the appropriate acyl chloride gave the bis-substituted anthraquinones in essentially quantitative yield. Structural assignments for the symmetrical products as **2a–n** are based on ^1H and ^{13}C-NMR spectra. Compound **2a** shows absorption for the H-4,8 protons at δ 8.16 (d, 2 H, J = 7.7 Hz), H-3,7 protons at δ 7.75 (t, 2 H, J = 8.0 Hz), and H-2,6 protons at δ 7.37 (d, 2 H, J = 8.1 Hz), respectively. Various reaction conditions and solvents were used and details are given in the experimental section. Furthermore, the ^{13}C-NMR spectra of these compounds showed the carbonyl resonance of anthraquinone chemical shift in the δ 181.1 region for C-9,10. The structures and biological activity of the symmetrical bis-substituted anthraquinones are listed in Table 1. Their *in vitro* cytotoxicity and lipid peroxidation properties are reported and compared with those of mitoxantrone, ascorbic acid, (+)-α-tocopherol, and starting material, respectively. The most active compounds displaying cytotoxicity was **2b**, **2f**, and **2n** with IC_{50} values 0.02, 0.04, and 0.4 μM against hep G2 cells, compared to mitoxantrone with IC_{50} value 2.0 μM, which indicated about 5 to 100 times the potency of the positive control, while lipid peroxidation was somewhat weaker than that of controls. The bis(butyryloxy) analogue **2b** demonstrates that the potent cytotoxicity could be regained without sacrificing potent antioxidant properties. The acyl substitution of **2b** leads to the highest activity against Hep G2 cell line when compared with mitoxantrone (about 100 times stronger); the bis-aromatic substitution of **2f** also shows good activity (about 50 times stronger); it is interesting that the differences between **2b** and **2n** are the end of substituted side chains, which are bis-butyroyloxy group and bis-(2-chlorobenzoyl) group, respectively. Moreover, bis(2-chlorobenzoyl) substituted as in **2f** even has slightly less cytotoxicity; however, the potency against lipid peroxidation was reduced. Introduction of phenylpropionyloxy groups into the 1,5-position linking the anthraquinone nucleus resulted in a decrease of cytotoxicity but the potent lipid peroxidation was strongly increased. Compounds **2n** exhibited stronger antioxidant activity than ascorbic acid, (+)-α-tocopherol and mitoxantrone. In contrast to (+)-α-tocopherol and ascorbic acid, **2a**, **2c**, **2d**, **2e**, and **2k** do not enhance lipid peroxidation in model membranes but exhibit prooxidation. The lipid peroxidation results show that not all members this series of compounds, except for **2n**, are good antioxidants; they may be promoters against lipid peroxidation. However, it is necessary to further investigate which **2n** was additionally evaluated at several concentrations against ascorbic acid and α-tocopherol with lipid peroxidation, giving the results shown in Table 2. At the concentration 0.01 mM, **2n** retained 50 % activity; the activity of ascorbic acid decreased to 10 %; α-tocopherol no longer had any more effect at 0.1 mM. It seems that **2n** is a better antioxidant against ascorbic acid and α-tocopherol. Based on this character, **2n** could be a potent antioxidant to protect normal cells from oxidative damage. By cross-linkage of the two biological evaluations, we can screen the compounds which are active in these evaluations. Among these compounds, **2n** is the most active one, both in its cytotoxicity and in its lipid-peroxidation.

Discussion

Based on the results of cytotoxic evaluation, we find that **2b**, with non-polar butyryl side chain at 1,5-positions, has the highest activity being 100 times stronger than mitoxantrone. The compound with the aromatic 2-chlorobenzoyl group, **2f**, also has good activity, being about 50 times stronger than mitoxantrone. Compared with **2b**, **2f**, and **2n**, the cytotoxicity of **2n** is lower although it is still 5 times stronger than that of mitoxantrone. Perhaps both lipophilic and van der Waals forces were needed to interact with the active site of Hep G2 cell line or helped to penetrate through the cell membrane; in addition, the

Table 1. Cytotoxicity against the growth of suspended murine and human tumors and inhibitory effect of anthraquinone derivatives on iron-induced lipid peroxidation in rat brain homogenates.



Compd	R	IC_{50} (μM)[a]		Inhibition of LP % (10 mM)[b]
		Hep G2[c]	C6 cells[d]	
2a	CH_2CH_3	4.1 ± 0.5	21.1 ± 1.6	−100
2b	$CH_2CH_2CH_3$	0.02 ± 0.01	38.5 ± 2.8	54 ± 2.2
2c	$CH_2CH_2CH_2CH_2CH_3$	36.2 ± 2.5	39.1 ± 4.1	−55 ± 1.5
2d	$C(CH_3)_3$	13.7 ± 1.8	12.0 ± 1.5	−23 ± 1.1
2e	C_6H_5	47.7 ± 5.5	38.7 ± 3.6	−50 ± 1.9
2f	2-ClC_6H_4	0.04 ± 0.01	40.7 ± 4.7	5 ± 0.5
2g	3-ClC_6H_4	15.1 ± 1.9	25.1 ± 2.8	1 ± 0.1
2h	4-ClC_6H_4	48.1 ± 4.5	38.6 ± 3.5	2 ± 0.2
2i	2,4-$Cl_2C_6H_3$	>50	38.4 ± 4.4	−1 ± 0.1
2j	2-$CH_3C_6H_4$	21.6 ± 2.2	25.1 ± 2.8	23 ± 1.1
2k	3-$CH_3C_6H_4$	18.1 ± 1.5	30.1 ± 3.3	−32 ± 1.5
2l	4-$CH_3C_6H_4$	9.3 ± 0.9	37.6 ± 4.1	33 ± 1.2
2m	$CH_2C_6H_5$	9.0 ± 1.5	39.1 ± 6.2	−1 ± 0.2
2n	$CH_2CH_2C_6H_5$	0.4 ± 0.1	40.1 ± 5.5	>100
	mitoxantrone	2.0 ± 0.5	0.07 ± 0.01	>100
	ascorbic acid			>100
	(+)-α-tocopherol			>100
	anthrarufin			−36 ± 1.1

[a] IC_{50}, drug concentration inhibiting 50 % of cellular growth following 48 h of drug exposure. Values are in μM and represent an average of three experiments. The variance for the IC_{50} was less than ±20 %. Inhibition of cell growth was significantly different with respect to that of the control; $N = 3$ or more, $P < 0.01$.

[b] Relative percentage of inhibition. Inhibition was compared to that of the control [ascorbic acid, >α-tocopherol and mitoxantrone-HCl], $P < 0.01$, Mean ± S.E., $n = 4$. Values are mean percent inhibition at the indicated concentration (mM), and standard errors.

[c] Hep G2: human hepatoma G2 cells.

[d] C6 cells: rat glioma C6 cells.

Table 2. Inhibitory effects of **2n** on iron-induced lipid peroxidation in rat brain homogenates.

Compound	Inhibition (%)[a]			
	10 mM	1 mM	0.1 mM	0.01 mM
2n	>100	>100	95 ± 2.0	50 ± 0.8
ascorbic acid	100	75 ± 1.5	32 ± 1.2	10 ± 0.6
(+)-α-tocopherol	100	55 ± 1.7	0	0
mitoxantrone-HCl	100	54 ± 2.1	22 ± 3.5	5 ± 0.3

[a] Relative percentage of inhibition. Inhibition was compared to that of the control [ascorbic acid, (+)-α-tocopherol and mitoxantrone-HCl], $P < 0.01$, Mean ± S.E., $n = 4$. Values are mean percent inhibition at the indicated concentration (mM), and standard errors.

distance between two forces may also be important. Substituted side chains of the three active compounds are obviously less polar than that of mitoxantrone. This means that on Hep G2 cell lines, the three non-polar side chains showed better affinities than polar side chains of mitoxantrone. Finally, **2 b**, **2 f**, and **2 n** are potent antitumor agent against human hepatoma cell line. In lipid peroxidation, **2 n** was the best antioxidant, even at a concentration 0.01 mM. It is a potent antioxidant protecting normal cells from oxidative damage, although the exact mechanism still needs to be investigated. The results of cytotoxic tests and lipid peroxidation show **2 n** to be the most active among these compounds. The interrelationships between results from the two tests of **2 n** are not very clear and it will be worthwhile undertaking further investigations.

Acknowledgements

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Experimental

Materials and apparatus

Melting points were determined with a Büchi B-545 melting point apparatus and are uncorrected. All reactions were monitored by TLC (silica gel 60 F_{254}), flash column chromatography: silica gel (E. Merck, 70–230 mesh) with CH_2Cl_2 as eluent. ^{1}H-NMR: Varian GEMINI-300 (300 MHz) and Brucker AM-500 (500 MHz); δ values are in ppm relative to TMS as an internal standard. Fourier-transform-IR spectra (KBr): Shimadzu FTIR-87000 spectrometer. UV spectra: Shimadzu UV-160A spectrometer. Mass spectra (EI, 70 eV, unless otherwise stated): Varian MAT 311A EIMS and Finnigan MAT 95 EIMS (Universität Regensburg).

Synthesis

General procedure for the preparation of 1,5-bisacyloxy anthraquinones

Method A: To a solution of anthrarufin (4.25 mmol) and pyridine (20 mL) in dry CH_2Cl_2 (150 mL) was added dropwise a solution of an appropriate acyl chloride (10 mmol) in dry CH_2Cl_2 (10 mL) at 0 °C under N_2. The reaction mixture was stirred or refluxed for 1–2 hours. Water (250 mL) was added and the mixture then extracted with dichloromethane. The combined organic extracts were washed with water and dried ($MgSO_4$), and concentrated. The resulting precipitate was collected by filtration, washed with water, and further purified by crystallization and chromatography.

Method B: To a solution of anthrarufin (4.25 mmol) in dry THF (20 mL) and NaH (12.75 mmol) was added dropwise a solution of an appropriate acyl chlorides (3 mmol) in dry THF (10 mL) at 0 °C under N_2. The reaction mixture was stirred or refluxed for 1–2 hours. Water (250 mL) was added and the mixture then extracted with dichloromethane. The combined organic extracts were washed with water and dried ($MgSO_4$), and concentrated. The resulting precipitate was collected by filtration, washed with water, and further purified by crystallization and chromatography.

1,5-Bis(propionyloxy)anthraquinone (**2 a**)

The title compound was obtained from **1** and acetyl chloride according to Method A. Recrystallization from ethanol gave yellow needles; 55% yield; mp 230–231 °C; ^{1}H-NMR ($CDCl_3$) δ 1.34 (t, J = 7.5 Hz, 6 H), 2.80 (q, J = 7.5 Hz, 4 H), 7.37 (d, J = 8.1 Hz, 2 H), 7.75 (t, J = 8.0 Hz, 2 H), 8.16 (d, J = 7.7 Hz, 2 H); FTIR (KBr): 1759, 1674 cm^{-1}; UV ν_{max} ($CHCl_3$) nm (log ε): 318 (2.48); MS *m/z* = 352 (4, M$^+$), 296 (23), 240 (100); Anal. $C_{20}H_{16}O_6$ (C, H).

1,5-Bis(butyryloxy)anthraquinone (**2 b**)

The title compound was obtained from **1** and butyryl chloride according to Method A. Recrystallization from ethanol gave yellow needles; 59% yield; mp 211–213 °C; ^{1}H-NMR ($CDCl_3$) δ 1.1 (t, J = 7.4 Hz, 6 H), 1.83–1.90 (m, 4 H), 2.75 (t, J = 7.5 Hz, 4 H), 7.36 (d, J = 8.0 Hz, 2 H), 7.74 (t, J = 7.9 Hz, 2 H), 8.16 (d, J = 7.8 Hz, 2 H); ^{13}C-NMR ($CDCl_3$) δ 181.14, 172.03, 150.08, 135.93, 134.88, 129.65, 125.63, 124.45, 36.15, 18.04, 13.75; UV ν_{max} ($CHCl_3$) nm (log ε): 319 (2.51); FTIR (KBr) 1757, 1676 cm^{-1}; MS *m/z* 380 (3, M$^+$), 310 (22), 240 (100); Anal. $C_{22}H_{20}O_6$ (C, H).

1,5-Bis(hexanoyloxy)anthraquinone (**2 c**)

The title compound was obtained from **1** and hexanoyl chloride according to Method A. Recrystallization from ethanol gave yellow needles; 74% yield; mp 183–184 °C; ^{1}H-NMR ($CDCl_3$) δ 0.95 (t, J = 7.1 Hz, 6 H), 1.38–1.49 (m, 8 H), 1.84 (q, J = 7.4 Hz, 4 H), 2.76 (t, J = 7.7 Hz, 4 H), 7.40 (dd, J = 7.8, 1.0 Hz, 2 H),7.74 (t, J = 8.1, 7.8 Hz, 2 H), 8.16 (t-like, J = 7.7, 2.3 Hz, 2 H); ^{13}C-NMR ($CDCl_3$) δ 181.14, 172.22, 150.10, 135.93, 134.87, 129.64, 125.63, 124.45, 34.26, 31.35, 24.18, 22.39, 13.97; UV ν_{max} ($CHCl_3$) nm (log ε) 318 (2.44); FTIR (KBr) 1755, 1676 cm^{-1}; MS *m/z* 436 (4, M$^+$), 338 (24), 240 (100); Anal. $C_{26}H_{28}O_6$ (C, H).

1,5-Bis(pivaloyloxy)anthraquinone (**2 d**)

The title compound was obtained from **1** and pivaloyl chloride according to Method B. Recrystallization from ethanol gave yellow needles; 25% yield; mp 166–167 °C; ^{1}H-NMR ($CDCl_3$) δ 1.47 (s, 18 H), 7.31 (d, J = 8.1 Hz, 2 H), 7.72 (d, J = 8.0 Hz, 2 H), 8.16 (d, J = 7.5 Hz, 2 H); ^{13}C-NMR ($CDCl_3$) δ 181.00, 176.66, 150.40, 135.98, 134.62, 129.38, 125.59, 124.78, 39.21, 27.23; UV ν_{max} (EtOH) nm (log ε) 363 (1.40); FTIR (KBr) 1751, 1670 cm^{-1}; MS *m/z* 408 (3, M$^+$), 324 (23), 240 (100); Anal. $C_{24}H_{24}O_6$ (C, H).

1,5-Bis(benzoyloxy)anthraquinone (**2 e**)

The title compound was obtained from **1** and benzoyl chloride according to Method A. Recrystallization from ethanol gave yellow needles; 76% yield; mp 336–338 °C (lit.[13] mp 342 °C); ^{1}H-NMR ($CDCl_3$) δ 7.50 (d, J = 7.9 Hz, 2 H), 7.56 (t, J = 7.7 Hz, 4 H), 7.68 (t, J = 7.3 Hz, 2 H), 7.77 (t, J = 7.9 Hz, 2 H), 8.17 (d, J = 7.7 Hz, 2 H), 8.29 (d, J = 7.7 Hz, 4 H); UV ν_{max} ($CHCl_3$) nm (log ε) 340 (0.69); FTIR (KBr) 1734, 1672 cm^{-1}; MS *m/z* 448 (4, M$^+$), 105 (100); Anal. $C_{28}H_{16}O_6$ (C, H).

1,5-Bis(2-chlorobenzoyl)anthraquinone (**2 f**)

The title compound was obtained from **1** and *o*-chlorobenzoyl chloride according to Method B. Recrystallization from THF gave yellow needles; 39% yield; mp 254–255 °C; ^{1}H-NMR ($CDCl_3$) δ 7.47–7.55 (m, 8 H), 7.80 (t, J = 7.9 Hz, 2 H), 8.22 (d, J = 7.8 Hz, 2 H), 8.39 (d, J = 7.7 Hz, 2 H); UV ν_{max} ($CHCl_3$) nm (log ε) 334 (2.20); FTIR (KBr) 1747, 1672 cm^{-1}; MS *m/z* 516 (2, M$^+$), 139 (100); Anal. $C_{28}H_{14}Cl_2O_6$ (C, H).

1,5-Bis(3-chlorobenzoyl)anthraquinone (**2 g**)

The title compound was obtained from **1** and *m*-chlorobenzoyl chloride according to Method B. Recrystallization from THF gave yellow needles; 49% yield; mp 301–302 °C; ^{1}H-NMR ($CDCl_3$) δ 7.50–7.52 (m, 4 H), 7.65 (d, *J* = 7.4 Hz, 2 H), 7.79 (t, *J* = 7.9 Hz, 2 H), 8.16–8.19 (m, 4 H), 8.26 (s, 2 H); UV ν_{max} ($CHCl_3$) nm (log ε) 351 (0.33); FTIR (KBr) 1744, 1674 cm^{-1}; MS *m/z* 516 (5, M$^+$), 141 (35), 139 (100); Anal. $C_{28}H_{14}Cl_2O_6$ (C, H).

1,5-Bis(4-chlorobenzoyl)anthraquinone (**2 h**)

The title compound was obtained from **1** and *p*-chlorobenzoyl chloride according to Method B. Recrystallization from THF gave yellow needles; 69% yield; mp 327–328 °C; ^{1}H-NMR ($CDCl_3$) δ 7.50 (d, *J* = 7.9 Hz, 2 H), 7.54 (d, *J* = 8.4 Hz, 4 H), 7.78 (t, *J* = 7.9 Hz, 2 H), 8.16 (d, *J* = 7.9 Hz, 2 H), 8.22 (d, *J* = 8.4 Hz, 4 H); UV ν_{max} ($CHCl_3$) nm (log ε) 351 (1.77); FTIR (KBr) 1736, 1676 cm^{-1}; MS *m/z* 516 (2, M$^+$), 139 (100); Anal. $C_{28}H_{14}Cl_2O_6$ (C, H).

1,5-Bis(2,4-dichlorobenzoyl)anthraquinone (**2 i**)

The title compound was obtained from **1** and *o,p*-dichlorobenzoyl chloride according to Method B. Recrystallization from THF gave yellow needles; 38% yield; mp 310–312 °C; IR (KBr) 1740, 1668 cm^{-1}; MS *m/z* 586 (4, M$^+$), 421 (25), 240 (56), 173 (100); HRMS *m/z:* Calcd. for $C_{28}H_{12}Cl_4O_6$: 514.1464. Found: 514.1478.

1,5-Bis(2-toluoyloxy)anthraquinone (**2 j**)

The title compound was obtained from **1** and *o*-toluoyl chloride according to Method A. Recrystallization from THF gave yellow needles; 68% yield; mp 262–263 °C; ^{1}H-NMR ($CDCl_3$) δ 2.68 (s, 6 H), 7.35 (d, *J* = 7.6 Hz, 2 H), 7.39 (t, *J* = 7.6 Hz, 2 H), 7.49–7.53 (m, 4 H), 7.78 (t, *J* = 7.9 Hz, 2 H), 8.19 (dd, *J* = 8.1, 0.1 Hz, 2 H), 8.35 (t, *J* = 7.7, 0.7 Hz, 2 H); ^{13}C-NMR ($CDCl_3$) δ 181.15, 165.55, 150.21, 141.57, 136.01, 134.93, 132.86, 131.91, 131.69, 129.89, 128.39, 126.03, 125.84, 124.71, 21.76; UV ν_{max} ($CHCl_3$) nm (log ε) 314 (1.56); FTIR (KBr) 1736, 1674 cm^{-1}; MS *m/z* 476 (2, M$^+$), 119 (100); Anal. $C_{30}H_{20}O_6$ (C, H).

1,5-Bis(3-toluoyloxy)anthraquinone (**2 k**)

The title compound was obtained from **1** and *m*-toluoyl chloride according to Method B. Recrystallization from THF gave yellow needles; 28% yield; mp 269–270 °C; ^{1}H-NMR ($CDCl_3$) δ 2.47 (s, 6 H), 7.44 (t, *J* = 7.6 Hz, 2 H), 7.48–7.50 (m, 4 H), 7.76 (t, *J* = 8.0 Hz, 2 H), 8.09 (d, *J* = 6.7 Hz, 4 H), 8.17 (d, *J* = 7.7 Hz, 2 H); ^{13}C-NMR ($CDCl_3$) δ 181.05, 165.28, 150.25, 138.52, 135.94, 134.91, 134.56, 130.96, 129.78, 129.34, 128.60, 127.67, 125.94, 124.60, 21.36; UV ν_{max} ($CHCl_3$) nm (log ε) 310 (1.84); FTIR (KBr) 1732, 1674 cm^{-1}; MS *m/z* 476 (4, M$^+$), 119 (100); Anal. $C_{30}H_{20}O_6$: (C, H).

1,5-Bis(4-toluoyloxy)anthraquinone (**2 l**)

The title compound was obtained from **1** and *p*-toluoyl chloride according to Method B. Recrystallization from THF gave yellow needles; 39% yield; mp 331–332 °C; ^{1}H-NMR ($CDCl_3$) δ 2.47 (s, 6 H), 7.36 (d, *J* = 8.0 Hz, 4 H), 7.49 (dd, *J* = 7.8, 1.0 Hz, 2 H), 7.75 (t, *J* = 7.9 Hz, 2 H), 8.17 (d, *J* = 7.8 Hz, 4 H); UV ν_{max} ($CHCl_3$) nm (log ε) 318 (0.90); FTIR (KBr) 1736, 1672 cm^{-1}; MS *m/z* 476 (5, M$^+$), 119 (100); Anal. $C_{30}H_{20}O_6$ (C, H).

1,5-Bis(phenylacetyloxy)anthraquinone (**2 m**)

The title compound was obtained from **1** and phenylacetyl chloride according to Method A. Recrystallization from THF gave yellow needles; 35% yield; mp 202–203 °C; ^{1}H-NMR ($CDCl_3$) δ 4.10 (s, 4 H), 7.30 (t, *J* = 7.4 Hz, 2 H), 7.33 (dd, *J* = 8.0, 0.8 Hz, 2 H), 7.37 (t, *J* = 7.6 Hz, 4 H), 7.46 (d, *J* = 7.4 Hz, 4 H), 7.74 (t, *J* = 8.0 Hz, 2 H), 8.18 (t, *J* = 7.8, 0.8 Hz, 2 H); ^{13}C-NMR ($CDCl_3$) δ 181.06, 170.12, 149.99, 135.87, 134.95, 133.33, 129.76, 129.57, 128.63, 127.31, 125.83, 124.28, 41.13; UV ν_{max} ($CHCl_3$) nm (log ε) 318 (2.20); FTIR (KBr) 1763, 1670 cm^{-1}; MS *m/z* 358 (5, M$^+$), 240 (94), 118 (100); Anal. $C_{30}H_{20}O_6$ (C, H).

1,5-Bis(phenylpropionyloxy)anthraquinone (**2 n**)

The title compound was obtained from **1** and phenylpropionyl chloride according to Method A. Recrystallization from THF gave yellow needles; 62% yield; mp 219–220 °C; ^{1}H-NMR ($CDCl_3$) δ 3.10 (s, 4 H), 3.17 (t, *J* = 7.9, 1.4 Hz, 4 H), 7.22–7.35 (m, 12 H), 7.74 (t, *J* = 7.9 Hz, 2 H), 8.16 (dd, *J* = 7.7, 0.8 Hz, 2 H); ^{13}C-NMR ($CDCl_3$) δ 181.08, 171.35, 149.97, 140.39, 135.88, 134.96, 129.62, 128.57, 128.47, 126.36, 125.75, 124.33, 35.86, 30.58; UV ν_{max} ($CHCl_3$) nm (log ε) 318 (1.10); FTIR (KBr) 1761, 1676 cm^{-1}; MS *m/z* 504 (5, M$^+$), 372 (10), 240 (100); Anal. $C_{32}H_{24}O_6$ (C, H).

Pharmacological studies

Cytotoxic evaluations (XTT colormetric assay)

Tumor cell lines used were rat glioma C6 cells and human hepatoma G2 cells. The cells (2.5×10^4 cells/mL) were placed into 96-well plates and preincubated for 24 to 72 hours in complete medium. The drug concentration inhibiting 50% of cellular growth (IC_{50}, mg/mL) was determined by XTT assay following 72 h of drug exposure. The results are the means of at least three independent experiments unless otherwise indicated.

Lipid peroxidation

Fresh S.D. rat brains were obtained and the residual vessels were cleaned up. The fresh brains were then homogenized with Kreb's buffer. After centrifugation, the upper solution (about 9 mL) was obtained. The 9 mL of solution was then separated between about 18 vials (500 mL/vial), which are divided into control and experimental sets. Then Kreb's buffer, 60 µL, and a DMSO solution of the tested compounds (30 µL), respectively, were added to the vials. After 10 minutes, ferrous sulfate solution was added to the control and experimental sets and maintained at 37 °C in water bath. After 30 minutes, the vials were taken from the water bath and trichloroacetic acid 10 mL (4% (*w/v*) in 0.3 N HCl) added to denature the residual protein. 2-Thiobarbituric acid solution 200 mL (0.5% (*w/v*) 2-thiobarbituric acid in 50% (*v/v*) acetic acid) was then added to the solution which was kept at 100 °C in a water bath for 15 minutes. The effects of the tested compounds on lipid peroxidation were determined by measuring the percentages of red-colored product formed by 2-thiobarbituric acid and malondialdehyde, which is one of the products formed by lipid peroxidation.

Statistics

The mean and standard deviation are designated as "$X \pm$ SD". The probable level of significance ($p \leq 0.05$) between test and control sample was determined by the Student's "t" test with the raw data.

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